# VirusExpress® 293 AAV Production Platform

## Draw on our experience to get on the fast track through production.



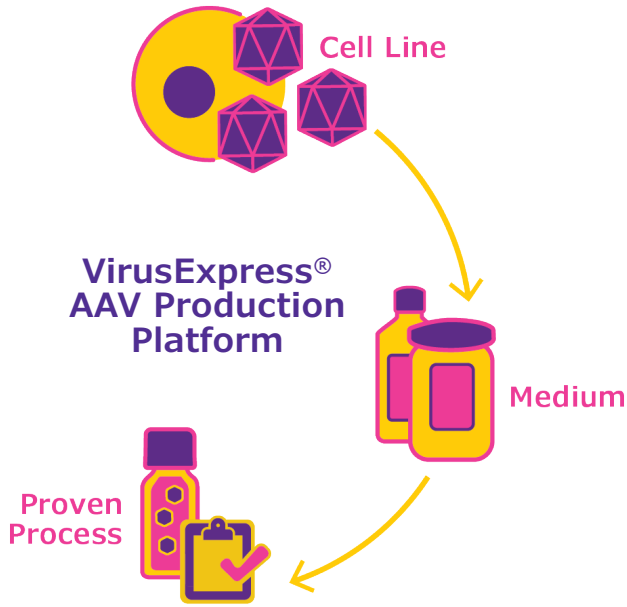
SAFC®

Pharma & Biopharma Raw Material Solutions

You're developing a life-saving gene therapy and need to manufacture adeno-associated viral (AAV) vector as quickly as possible.

# We're here to help.

The VirusExpress® AAV Production Platform offers a transfection-based solution using a suspension adapted cell line, chemically defined medium, and a clinically relevant process dramatically reducing time to commercial production. Find out how our 30+ years of viral vector experience can help bring your gene therapy to life.




## VirusExpress® AAV Platform

The challenges faced by AVV manufacturers can be alleviated with a scalable AAV manufacturing offering. The VirusExpress® AAV platform includes: optimized medium for transient transfection, suspension-adapted cells, and bioreactor process development and scale-up. The VirusExpress® 293 AAV Production cells have been single-cell cloned and have been suspension adapted into chemically defined cell culture medium (EX-CELL® CD HEK293 Viral Vector Medium, 14385C). The cell bank was manufactured and characterized under GMP regulations (21 CFR 210, 211, 600, and 610). And the process development has been proven up to 3L production scale as well as cell growth up to 50 L scale.

## VirusExpress® 293 Cell Growth and Viability in 50 L and 3 L Mobius® Single-use Bioreactors

Our approach for early bioreactor process development was to divide the development into two phases. Phase I focused on developing robust cell growth parameters. Phase II was designed to focus on optimizing the transfection conditions for optimal AAV2 production. The rationale for the division was similar to approach for the VirusExpress® 293 Lentivirus Platform. We wanted to ensure we developed the appropriate protocols for cell growth and cell health to avoid adversely affecting the cells production capacity.

The suspension cell culture conditions have been established using GMP cell culture media. The cell growth (density) and viability profiles were assessed at shake flask, 3 L Mobius® Single-use Bioreactor and 50 L Mobius® Single-use Bioreactor scales. The process development scientists have established robust protocols to ensure the target growth rates are achievable and reproducible at clinically significant scales for optimal viral production conditions. The viable cell density (VCD) met or exceeded the target density of $8x10^6$ cells/mL at all scales.

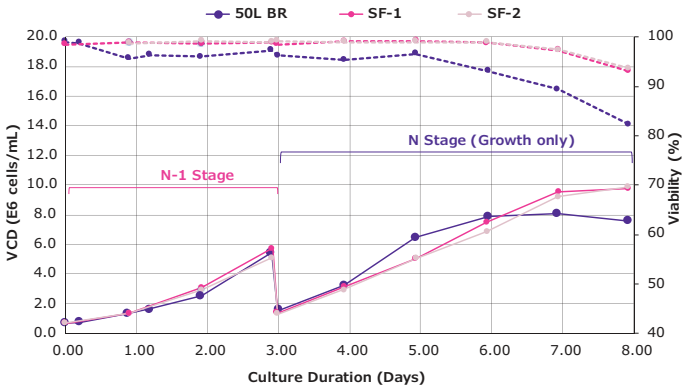



**Figure 1**: Cell Growth and Viability in 50 L Mobius® Single-use Bioreactor compared to Shake Flasks

VCD in the 50 L bioreactor as shown by the solid black line met the target of 8x10⁶ cells/mL for the N Stage growth at day 6 as well as well as >90% viability as shown by the black dashed line after day 6 to demonstrate the scalability of the platform versus the shake flask controls which are represented by the blue lines.

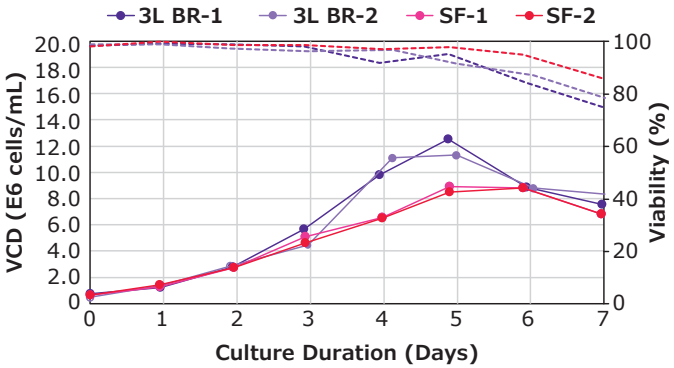



**Figure 2**: Cell Growth and Viability in 3 L Mobius® Single-use Bioreactors compared to Shake Flasks

VCD in the 3 L bioreactor as shown by the solid purple lines exceeded the target of 8x10⁶ cells/mL in 3 L bioreactors for growth at day 5 as well as well as >95% viability as shown by the dashed purple lines after 5 days to demonstrate the scalability of the platform versus the shake flask controls which are represented by the red lines.

## VirusExpress® AAV Baseline Process

The process development scientists have been able to optimize the triple plasmid transfection conditions at clinically relevant scale in a 3 L Mobius® Single-use Bioreactor (2 L working volume) to produce AAV2 genome titer above the target titer of 1x10⁹ gc/mL. Consistently, the scientists achieved higher genome titers in bioreactors as compared to shake flasks. Our baseline process was able to reach 9x10⁹ gc/mL, which nearly is a log higher than the target titer for the AAV2 process. The simplified upstream workflow is shown in **Figure 3**.

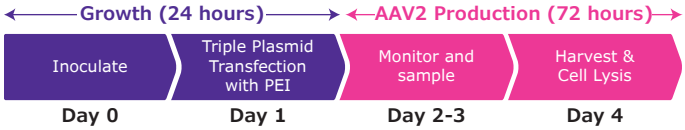



**Figure 3:** Schematic of the upstream process flow and associated timing for AAV production.

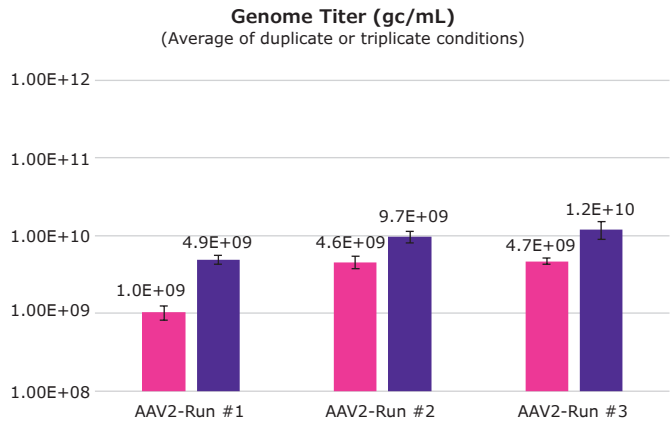
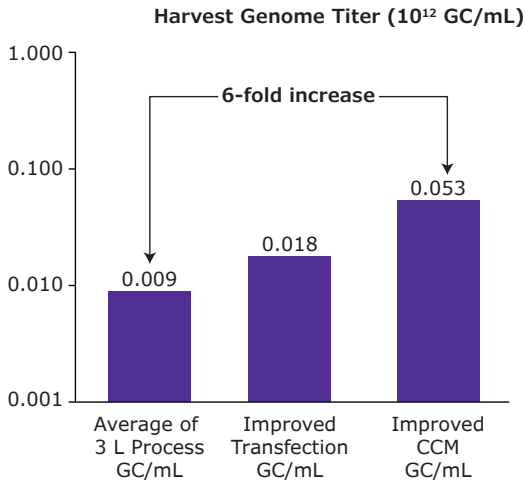



**Figure 4**: VirusExpress® Platform - Baseline Process for AAV 2 Production in 3 L Mobius ® Single-use Bioreactors. AAV2 crude harvest genome titers (gc/mL) of GFP model virus. Bioreactor data is shown in purple, and shake flask data is shown in pink.

Genome titers are consistently higher in 3 L Mobius® Single-use Bioreactor than shake flask. The genome titers exceeded the target of 1x10⁹ gc/mL for all 3 production runs as shown by the purple bars for an average of 9x10⁹ gc/mL of the 3 runs versus shake flask controls represented by the magenta bars.

With process improvements planned for not only transfection reagent optimization but also for cell culture media conditions, the process development scientists have seen at least a 6-fold increase for AAV2 genome titers as compared to the average 3 L baseline process. Mobius® Single-use Bioreactor process from 9x10⁹ to 5x10¹⁰ gc/mL as shown in **Figure 5**.




6X

**VirusExpress® AAV Performance Increase**

- Transfection agent
- Cell Culture Media

**Figure 5:** Planned performance enhancements will achieve at least a 6-fold increase in genome titers from 9x10⁹ gc/mL to 5x10¹⁰ gc/mL for AAV2 production.

## VirusExpress® Production Platform Performance in Multiple Serotypes

Not only have the process development scientist been able to optimize the AAV2 production process, but also the scientists have shown the cell line is amendable to production of GFP model virus in AAV5 and AAV6 serotypes in shake flasks using our baseline process conditions.

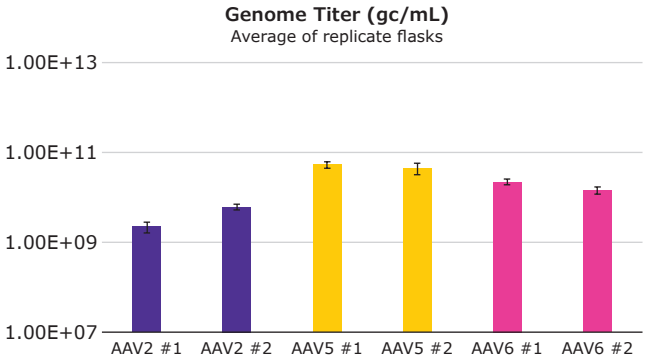


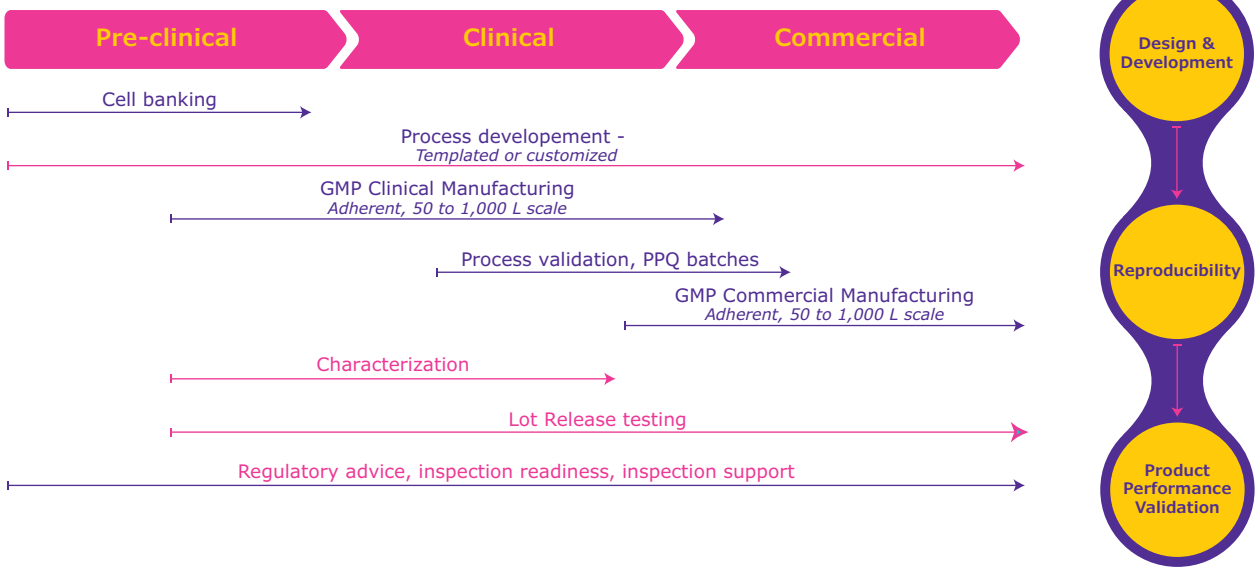

**Figure 6**: VirusExpress® Production compared across multiple serotypes in Shake flasks shown as genome titers (gc/mL) of GFP model virus.

Shake flask production comparison of AAV2, AAV5 and AAV6 genome titers shows the robustness of the VirusExpress® Production Platform.

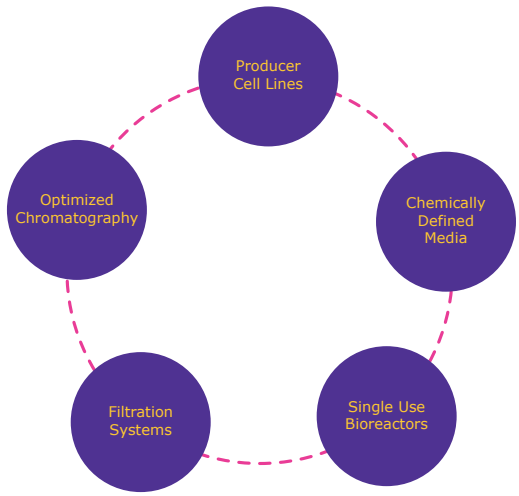
## Our Cell Culture Medium for Simplified Feedstream

EX-CELL® CD HEK293 Viral Vector Medium is formulated to support high suspension cell densities; high transient transfection efficiency when used with polyethyleneimine (PEI) as the transfection reagent; and high viral titers in shake flask and stirred tank bioreactor formats. In addition to PEI the medium also supports lipid polymer-based transfection protocols and cationic polymer-based transfection protocols. This is a simplified solution for feedstream cell culture medium (CCM) which does not require a post-transfection exchange, enhancer, boost or feed when used in a batch production process as part of the VirusExpress® 293 AAV Production Platform. Also, this CCM is suitable as a stand-alone solution for other HEK293 and HEK 293T cell lines used for AAV, lentivirus, and adenovirus manufacturing.

# Process Development Services: How We Can Help




Process Development (PD) is central from early stage into clinical manufacturing, in preparation to commercializing through the product lifecycle. PD is involved from the design and development of a cell line and process, to reproducibility in manufacturing, and to commercialization readiness and execution. We are dedicated to supporting your needs from pre-clinical to commercial, and you can count on our 30+ years of gene therapy manufacturing experience to help meet your gene therapy needs.




Our PD scientist along with the deep integrated solutions offerings that we provide for viral vector manufacturing allows us to provide templated solutions and key value drivers for our customers to speed their therapies to market, reduce risk, and lower process costs.

## Key Value Drivers

### Risk Reduction

Chemically defined, animal component free CC media. GMP banked and characterized cells. Scalable, proven process.

### Clinical Speed

Utilizing process development expertise, clinical manufacturing in as little as 6 months.

### Process costs

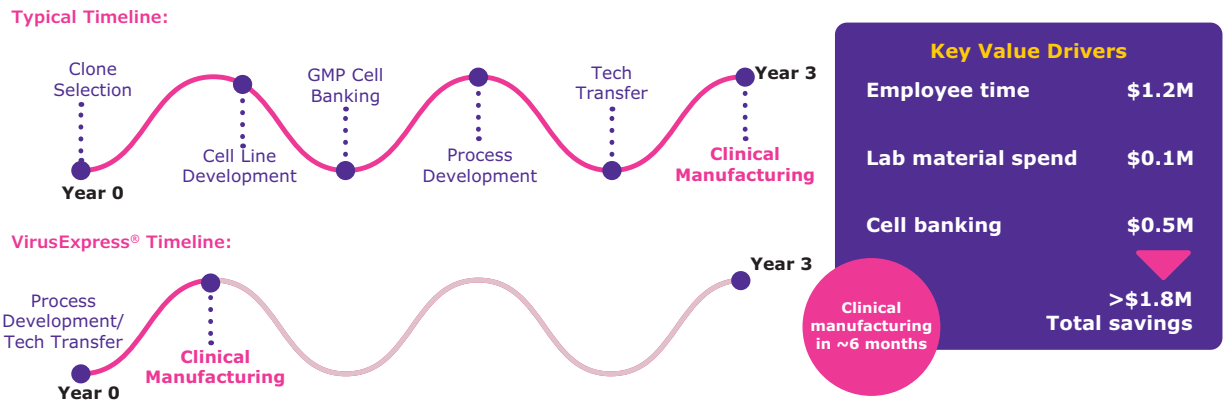
Lower operational spend enabled by more productive runs (increased titers).

## BioReliance® Viral and Gene Therapy Manufacturing Capabilities: We Are Here

We have the right cell lines, media products, manufacturing technologies and expertise to meet our customers' timelines and their patients' needs. By utilizing the VirusExpress® Production Platform, we estimate you may be able to begin clinical manufacture in as little as 6 months as opposed to ~3 years for a custom cell line and process since clone selection, cell line development and GMP cell banking are complete, as well as substantial process development and large-scale bioreactor development. Leveraging our expertise in viral vector manufacturing as well as utilizing our templated process solutions will help deliver your gene therapy to patients faster.

## Our BioReliance® Viral and Gene Therapy Manufacturing and Process Development Services Offering

### Standardizing Manufacturing Timelines with VirusExpress®

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